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Nitrogen balance in lambs fed low-quality brome hay and infused with differing proportions of casein in the rumen and abomasum^{1,2}

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ABSTRACT: Twenty wether lambs (46 ± 2 kg) fitted with ruminal and abomasal infusion catheters were used in a completely randomized design to determine the effects of differing proportions of ruminal and abomasal casein infusion on N balance in lambs fed low-quality brome hay (0.8% N, DM basis) for ad libitum intake. Wethers were infused with 0 (control) or 10.7 g/d of N from casein with ratios of ruminal:abomasal infusion of 100:0 (100R:0A), 67:33 (67R:33A), 33:67 (33R:67A), or 0:100% (0R:100A), respectively, over a 12-d period. Total N supply (hay N intake + N from casein infusion) was greater ($P = 0.001$) in lambs receiving casein infusion than in controls. Urinary N excretion (g/d) was greater ($P = 0.001$) in lambs receiving casein infusion than in controls. Urinary N excretion decreased as casein infusion was shifted from 100R:0A to 33R:67A and then slightly increased in lambs receiving 0R:100A (quadratic, $P = 0.02$). Total N excretion was greater ($P = 0.001$) in lambs receiving casein infusion than in controls and decreased linearly ($P = 0.005$) as casein infusion was shifted to the abomasum. Retained N (g/d, % of N intake, and % of digested N) was greater ($P = 0.001$) in lambs receiving casein than in

controls. Retained N increased as infusion was shifted from 100R:0A to 33R:67A and then slightly decreased in lambs receiving 0R:100A (quadratic, $P < 0.07$). Based on regression analysis, the predicted optimum proportion of casein infusion to maximize N retention was 68% into the abomasum. The regression suggests that supplementation with undegradable intake protein had an additional benefit over supplementation with ruminally degradable intake protein (100R:0A) and that changing the percentage of ruminally undegradable intake protein in supplemental protein from 33 to 100% resulted in minimal differences in N retention. Apparent N, DM, OM, and energy digestibility (% of intake) was greater ($P < 0.03$) in lambs infused with casein than controls but did not differ among casein infusion groups. These data suggest that feeding protein supplements containing a portion (greater than 0%) of the crude protein as ruminally undegradable intake protein, as compared to 100% ruminally degradable intake protein, to lambs consuming low-quality forage increases N retention and the efficiency of N utilization without influencing total-tract nutrient digestion.

Key Words: Nitrogen Metabolism, Nutrient Balance, Sheep, Supplementation

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Introduction

Forage quality changes with season (Johnson et al., 1998) and often is not of adequate quality to maintain acceptable performance of grazing ruminants. Supplemental CP often increases weight gain (or decreases

weight loss) of ruminants fed or grazing low-quality forages (Owens et al., 1991). It is generally considered that ruminally degradable intake protein (**DIP**) supplementation of ruminants fed low-quality forages is necessary to improve forage digestibility and increase intake. However, performance of ruminants fed ruminally undegradable intake protein (**UIP**) supplements often improves or does not differ from ruminants fed DIP supplements (Schloesser et al., 1993; Alderton et al., 2000; Bohnert et al., 2002). Site of protein digestion can be an important factor determining metabolizable protein supply. However, when comparing sources of supplemental protein with differing protein degradability, it can be difficult to ascertain whether changes in the efficiency of N utilization are the result of changes in site of digestion or protein quality (digestibility and amino acid composition). By using a common protein source (casein) and infusing different proportions in the

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Table 1. Analysis of brome hay and casein infusate

Analysis	Brome hay	Casein infusate
DM, % of as-fed	93.6	5.7
Ash, % of DM	8.0	4.3
N, % of DM	0.8	15.3
Gross energy, kcal/g DM	4.3	5.4
Cell contents, % of DM	26.1	100
NDF, % of DM	73.9	—
ADF, % of DM	48.1	—
Cellulose, % of DM	37.1	—
Lignin, % of DM	6.5	—

Table 2. Composition of supplemental mineral mix^a

Ingredient	% of DM
Sodium chloride	22.21
Dicalcium phosphate	44.45
MgSO ₄ ·7H ₂ O	26.68
Vitamin A, D, and E premix ^b	2.22
Vitamin E premix ^c	2.22
Trace mineral premix ^d	2.22

^aFed to wethers at 20 g/d (DM basis).^bEach gram of premix contained (DM basis) 8,800 IU of vitamin A, 880 IU of vitamin D, and 0.88 IU of vitamin E.^cEach gram of premix contained (DM basis) 44 IU of vitamin E.^dContained (DM basis) 14% Ca, 12% Zn, 8% Mn, 10% Fe, 0.2% I, and 0.1% Co.

rumen and abomasum, we can examine the effect of site of protein digestion without changes in protein quality. Our goal was to examine the effect of changing the proportion of supplemental protein (casein) that is digested in the rumen and abomasum on N balance in wethers fed low-quality brome hay.

Materials and Methods

Animals and Abomasal Infusion Treatments

The experiment was approved by the U.S. Meat Animal Research Center Animal Care and Use Committee and complied with the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). Twenty wether lambs (46 ± 2 kg; 10 polled Dorset \times Suffolk, five polled Dorset \times Rambouillet, and five polled Dorset) were housed in individual 1.17×1.17 -m pens before the experimental periods began. Infusion catheters were placed in the rumen and abomasum of lambs under general anesthesia to facilitate casein infusion ruminally and postruminally, respectively (Gross et al., 1990). Lambs were withheld from feed and water for 48 and 24 h, respectively, before surgery. General anesthesia was induced with sodium pentothal and maintained with halothane. Lambs had been used in a previous experiment (Swanson et al., 2004).

Wethers were offered chopped brome hay (Table 1; ground with a tub grinder equipped with a 10-cm screen) to appetite, and each wether received a loose mineral mix at 20 g/d (Table 2). Wethers were placed in metabolism crates during experimental periods. There were two experimental periods in which 10 wethers were used in each period. Wethers were randomly assigned within breed to one of five experimental treatments. Wethers were infused with 0 (control) or 70.0 ± 0.8 g/d sodium caseinate (Table 1; 10.7 ± 0.1 g N/d; New Zealand Milk Products, Santa Rosa, CA) with ratios of ruminal:abomasal infusion of 100:0 (**100R:0A**), 67:33 (**67R:33A**), 33:67 (**33R:67A**), and 0:100 (**0R:100A**). A total of $1,225 \pm 6$ g and $1,229 \pm 6$ g of casein solution (or water) were infused into the rumen and abomasum, respectively, per day. Infusion of casein solution or water was accomplished with a peristaltic pump (Harvard

Apparatus Model 1217, South Natick, MA) and was continuous during the 12-d periods (described below). A 5.7% (DM basis) stock casein solution was made, stored frozen, and diluted as necessary, depending on treatment, prior to use. Casein was used as the protein source because of its high ruminal degradability and small intestinal digestibility. Infusion periods were 12 d in length. Before infusion periods, lambs were adapted to the brome hay and were supplemented with 110 g/d of soybean meal for at least 3 wk. One week prior to the initiation of infusion treatments, soybean meal supplementation was discontinued for lambs on the control treatment and continued for the casein infusion treatments until the initiation of the infusion treatments. This was done to provide adequate adjustment time for the lambs on the control treatment and should have allowed the lambs to be closer to a steady state by the collection period. Days 1 through 7 of the infusion periods were for adaptation to the infusion treatment. On d 8 through 12, orts, feces, and urine were collected and composited (100, 50, and 5% of orts, feces, and urine; weight basis) within animal and stored at -30°C for N balance measurements. Feces were collected into fecal bags to avoid mixing of feces and urine. Urine was collected into plastic bottles containing 100 mL of 4 M HCl to maintain urine pH <3 . Composited feed, orts, and feces were dried in a forced-air oven (55°C) and ground (1-mm screen) using a Wiley Mill (Arthur Thomas Co., Philadelphia, PA). Concentrations of OM (AOAC, 1990), N (Leco FP-2000, Leco Corporation, St. Joseph, MI), NDF (Robertson and Van Soest, 1981), and gross energy (bomb calorimetry) were determined on feed, orts, casein infusion, and fecal samples to determine apparent total-tract digestibility. Also, on d 8 and 12, blood samples were collected at 1500 into heparinized syringes, plasma harvested by centrifugation, stored at -30°C , and later analyzed for urea N (Marsh and Fingerhut, 1965) using a Technicon Autoanalyzer (Method #339-01; Technicon Autoanalyzer System, Tarrytown, NY). On d 8, a temporary catheter (Tygon microbore tubing, 1.02-mm inner diameter, 1.78-mm outer diameter, 0.38-mm wall thickness) was placed in a jugular vein to facilitate blood collection.

Statistical Analyses

All data, except plasma urea N concentrations, were analyzed as a randomized block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included abomasal infusion period and treatment. Plasma urea N concentrations were analyzed using the repeated statement with the mixed procedure of SAS. The model included period, treatment, day, and treatment \times day. Animal within treatment was used as the SUBJECT and autoregression was used as the covariance structure. For all analyses, treatment means were compared using orthogonal contrast statements. Orthogonal contrasts were control vs. the mean of the casein infusion treatments and linear, quadratic, and cubic effects of site of casein infusion. Differences were considered significant when $P < 0.10$.

Results

Intake of hay N did not differ between treatments (Table 3), but total N supply (hay N intake + N from casein infusion) was greater ($P = 0.001$) in lambs receiving casein infusion than controls. Urinary N excretion (g/d) was greater ($P = 0.001$) in lambs receiving casein infusion compared to controls. Urinary N excretion decreased as casein infusion was shifted from 100R:0A to 33R:67A and then slightly increased in lambs receiving 0R:100A (quadratic, $P = 0.02$). However, fecal N excretion did not differ between treatments. Total N excretion was greater ($P = 0.001$) in lambs receiving casein infusion compared to controls and decreased linearly ($P = 0.005$) as casein infusion was shifted to the abomasum. Urinary urea N excretion (g/d) was greater ($P = 0.001$) in lambs receiving casein infusion compared to controls. Urinary urea N excretion decreased as casein infusion was shifted from 100R:0A to 67R:33A, did not change between 67R:33A and 33R:67A, and then slightly increased in lambs receiving 0R:100A (quadratic, $P = 0.06$). Urinary non-urea N excretion (g/d) did not differ between treatments. Apparently digested N (g/d and % of N intake) was greater ($P < 0.001$) in lambs infused with casein than controls, but did not differ between casein infusion treatments. Retained N (g/d, % of N intake, and % of digested N) increased as casein infusion was shifted from 100R:0A to 33R:67A and slightly decreased in lambs receiving 0R:100A (quadratic, $P < 0.07$). No treatment \times day interactions were observed for plasma urea N concentrations. Plasma urea N concentration was greater ($P = 0.001$) in lambs receiving casein infusion compared to controls. Plasma urea N concentration decreased as casein infusion was shifted from 100R:0A to 33R:67A and then slightly increased in lambs receiving 0R:100A (quadratic, $P = 0.03$).

Average daily gain did not differ between treatments. However, lambs receiving casein had numerically greater ADG (-0.21 , -0.03 , -0.04 , and -0.05 kg/d for 100R:0A, 67R:33A, 33R:67A, and 0R:100A, respec-

tively) than controls (-0.27 kg/d, SEM = 0.05; data not shown). Hay intake and total supply of DM, OM, energy, and NDF were not significantly influenced by treatment even though intakes increased 14 to 60% in lambs infused with casein relative to controls (Table 4). Fecal DM, OM, NDF, and energy excretion were not influenced by treatment. Apparently digested DM, OM, and energy (g/d or Mcal/d) were greater ($P < 0.03$) in lambs receiving casein infusion than controls but did not differ between casein infusion treatments. Apparent DM, OM, and energy digestibility (% of intake) was greater ($P < 0.003$) in lambs infused with casein than controls but did not differ between casein infusion groups. Apparent digestion (g/d and % of intake) of NDF did not differ between treatments.

Discussion

Protein supplementation is often necessary to optimize production. Site of protein digestion is an important component associated with metabolizable protein supplied to the animal. However, changes in protein quality among supplements make it difficult to ascertain whether differences in performance are the result of differences in the site of protein digestion or the quality. Our objective was to specifically examine the effect of site of protein digestion on N balance in lambs fed low-quality brome hay.

The brome hay used in this experiment was of low quality as noted by the high fiber, low N concentration, and low nutrient digestibility observed. Apparent digestibility of hay N (% of supply) by control lambs was very low (9.4%). This low apparent digestibility may be partly because of the excretion of metabolic fecal N (endogenous N). The hay alone was not adequate to maintain performance as N retention and ADG were negative for lambs on the control treatment. Infusion of casein helped to overcome this deficiency as N retention (grams) became positive and ADG numerically approached zero. A quadratic effect of site of casein infusion was observed with the greatest retention (grams) and efficiency of retention (% of N intake and % of digested N) in lambs receiving 33R:67A. Based on regression analysis (Figure 1) using all data points, the optimum proportion of casein infusion to maximize N retention is predicted to be 68% into the abomasum. The regression (Figure 1) suggests that UIP supplementation had an additional benefit over just DIP supplementation (100R:0A) and that changing the percentage of UIP in supplemental protein from 33 to 100% resulted in minimal differences in N retention. Sources of supplemental protein commonly used for forage-based diets contain from approximately 35 to 90% UIP (NRC, 1996). It is not surprising that differences in N retention are often not observed (Schloesser et al., 1993; Alderton et al., 2000; Bohnert et al., 2002) when comparing natural protein supplements differing in ruminal degradability, unless the quality of the supplemental protein sources differ (i.e., differences in digestibility or amino

Table 3. Influence of infusing different proportions of casein in the rumen and abomasum on N balance and plasma urea N concentrations in lambs fed low-quality brome hay^a

Item	Treatment ^b						<i>P</i> = ^d	Contrast <i>P</i> = ^c			
	Control	100R:0A	67R:33A	33R:67A	0R:100A	SEM		Control vs. others	Linear site	Quadratic site	Cubic site
N intake, g/d											
Hay	3.90	4.31	5.28	4.31	4.33	0.66	0.67	0.39	0.77	0.51	0.37
Infusion	0	10.77	10.72	10.71	10.71	0.08	0.001	0.001	0.65	0.77	0.96
Total	3.90	15.08	16.00	15.02	15.04	0.64	0.001	0.001	0.71	0.51	0.35
N excretion, g/d											
Urine	2.14	10.89	8.67	8.01	8.20	0.42	0.001	0.001	0.002	0.02	0.73
Feces	3.50	4.15	4.95	4.12	4.07	0.46	0.33	0.13	0.62	0.39	0.28
Total	5.64	15.04	13.62	12.13	12.27	0.61	0.001	0.001	0.005	0.24	0.56
Urinary urea N excretion g/d	0.60	9.68	7.14	7.14	7.69	0.67	0.001	0.001	0.10	0.06	0.56
% of total urinary N	27.5	88.7	82.8	89.5	92.5	5.93	0.001	0.001	0.55	0.51	0.59
Urinary non-urea N excretion g/d	1.54	1.21	1.53	0.87	0.51	0.51	0.39	0.39	0.30	0.57	0.62
% of total urinary N	72.5	11.3	17.2	10.5	7.5	5.93	0.001	0.001	0.55	0.51	0.59
N excretion, % of total N excretion											
Urine	37.9	72.4	64.2	66.0	67.2	2.86	0.001	0.001	0.30	0.13	0.44
Feces	62.1	27.6	35.8	34.0	32.8	2.85	0.001	0.001	0.30	0.13	0.42
N excretion, % of N intake											
Urine	56.2	72.6	54.4	53.7	54.8	4.54	0.05	0.60	0.01	0.04	0.42
Feces	90.6	27.6	30.5	27.2	26.6	1.99	0.001	0.001	0.51	0.40	0.35
Apparent N digested g/d	0.40	10.93	11.05	10.90	10.97	0.29	0.001	0.001	0.98	0.93	0.74
% of N intake	9.4	72.4	69.5	72.8	73.4	2.51	0.001	0.001	0.51	0.40	0.35
N retained g/d	-1.75	0.04	2.39	2.89	2.77	0.53	0.001	0.001	0.008	0.06	0.66
% of N intake	-46.76	-0.14	15.10	19.06	18.58	4.60	0.001	0.001	0.003	0.05	0.68
% of digested N	-684.59	-0.56	21.49	26.30	25.38	96.5	0.001	0.001	0.004	0.05	0.62
Plasma urea N, mM	1.41	10.65	8.28	7.53	7.76	0.48	0.001	0.001	0.003	0.03	0.79

^aValues are least squares means and pooled SEM, *n* = 4.

^bWethers were infused with 0 (control) or 10.7 g/d of N from casein with ratios of ruminal:abomasal infusion of 100:0 (100R:0A), 67:33 (67R:33A), 33:67 (33R:67A), or 0:100% (0R:100A) over a 12-d period.

^cControl vs. others = control vs. the mean of 100R:0A, 67R:33A, 33R:67A, or 0R:100A; linear site, quadratic site, cubic site = linear, quadratic, and cubic effects of site of casein infusion, respectively.

^d*P* = observed significance level for the main effect of abomasal infusion treatment.

acid profile). The lower N retention observed for lambs on the 100R:0A treatment compared to other casein infusion treatments also could help explain why ruminants receiving natural protein supplements often perform better than ruminants receiving supplemental urea (100% DIP; Owens et al., 1991). It also suggests that the requirement for DIP in this experiment was low because of the limited amount of fermentable carbohydrate available in the rumen and the limited ability of the microbial population to incorporate ammonia into microbial protein or because of high levels of recycled urea N contributing to total ruminal available nitrogen levels in lambs receiving casein infusion.

It has been suggested that urea N recycling might play a key role when UIP is supplemented to ruminants consuming low-quality forage (Bandyk et al., 2001; Bohnert et al., 2002). Kennedy and Milligan (1980) noted that urea N recycling to the gastrointestinal tract is associated with ruminal ammonia concentration, plasma urea concentrations, and the amount of organic

matter digested in the rumen. Ruminal ammonia concentrations, although not measured, likely decreased as casein infusion was shifted to the abomasum, possibly making increases in urea N recycling more probable. However, plasma urea N concentrations were reduced in lambs receiving abomasal casein (67R:33A, 33R:67A, and 0R:100A compared with 100R:0A) in this experiment, and limited amounts of fermentable OM in all treatments may have limited the ability of the rumen to “pull” urea from the blood for utilization and incorporation into microbial protein. Therefore, although recycled urea N is likely an important component of total available N in the rumen, the role that urea N recycling had on observed changes in N retention is unclear.

Intake of low-quality forage often increases with protein supplementation (Kartchner, 1981; McCollum and Galyean, 1985; Krysl et al., 1987) presumably because of improved digestion of fiber and increased passage rate or because of metabolic signals associated with postruminal amino acid flow. Forage DM intake was

Table 4. Influence of infusing different proportions of casein in the rumen and abomasum on DM, OM, and energy intake and apparent digestibility in lambs fed low-quality brome hay^a

Item	Treatment ^b					SEM	<i>P</i> = ^d	Contrast <i>P</i> = ^c			
	Control	100R:0A	67R:33A	33R:67A	0R:100A			Control vs. others	Linear site	Quadratic site	Cubic site
Intake											
DM, g/d											
Hay	469	537	661	542	560	84.1	0.62	0.28	0.90	0.58	0.36
Infusion	0	70	70	70	70	0.48	0.001	0.001	0.64	0.77	0.96
Total	469	607	731	612	630	83.9	0.34	0.08	0.90	0.56	0.36
OM, g/d											
Hay	431	496	613	504	520	77.0	0.59	0.25	0.92	0.55	0.36
Infusion	0	67	67	67	67	0.46	0.001	0.001	0.64	0.77	0.96
Total	431	563	680	571	587	76.8	0.30	0.07	0.91	0.55	0.35
Energy, Mcal/d											
Hay	2.01	2.33	2.86	2.37	2.43	0.36	0.59	0.25	0.91	0.55	0.37
Infusion	0	0.38	0.38	0.38	0.38	0.003	0.001	0.001	0.64	0.77	0.96
Total	2.01	2.71	3.24	2.75	2.81	0.36	0.25	0.05	0.91	0.55	0.37
NDF, g/d	364	426	494	411	442	65.9	0.73	0.30	0.92	0.80	0.43
Fecal excretion											
DM, g/d	289	306	389	323	319	43.4	0.57	0.37	0.89	0.32	0.29
OM, g/d	249	263	335	279	275	38.3	0.58	0.38	0.90	0.32	0.29
Energy, Mcal/d	1.25	1.35	1.70	1.42	1.37	0.19	0.53	0.34	0.79	0.29	0.29
NDF, g/d	206	215	276	231	224	33.0	0.62	0.42	0.91	0.31	0.32
Apparent digestibility											
DM											
g/d	180	302	343	289	311	45.2	0.17	0.02	0.91	0.85	0.46
% of intake	38.3	49.1	49.6	47.8	49.7	2.30	0.02	0.002	0.75	0.32	0.76
OM											
g/d	181	301	345	292	312	42.8	0.13	0.02	0.93	0.81	0.44
% of intake	42.1	52.9	50.6	51.8	53.7	2.25	0.02	0.001	0.69	0.32	0.77
Energy											
Mcal/d	0.76	1.36	1.54	1.33	1.44	0.19	0.08	0.008	0.98	0.88	0.48
% of intake	37.8	49.9	47.4	49.1	51.6	2.17	0.004	0.001	0.45	0.22	0.68
NDF											
g/d	158	212	218	181	218	38.3	0.75	0.28	0.93	0.73	0.56
% of intake	43.9	48.7	43.6	44.6	48.6	4.37	0.69	0.52	0.96	0.18	0.84

^aValues are least squares means and pooled SEM, *n* = 4.^bWethers were infused with 0 (control) or 10.7 g/d of N from casein with ratios of ruminal:abomasal infusion of 100:0 (100R:0A), 67:33 (67R:33A), 33:67 (33R:67A), or 0:100% (0R:100A) over a 12-d period.^cControl vs. others = control vs. the mean of 100R:0A, 67R:33A, 33R:67A, or 0R:100A; linear site, quadratic site, cubic site = linear, quadratic, and cubic effects of site of casein infusion, respectively.^d*P* = observed significance level for the main effect of abomasal infusion treatment.

only about 1.2% of BW in this experiment and did not statistically differ between treatments, although a numerical trend for increased intake (23%) in lambs infused with casein was evident and tended to be greater in lambs receiving 67R:33A compared to controls (contrast statement *P* = 0.11; data not shown). However, others also have reported no differences in forage intake when supplemented with protein (Rittenhouse et al., 1970; Ferrell et al., 1999; Bohnert et al., 2002).

If one assumes that casein was 100% digestible, calculated increases in forage DM digestibility were 4.9, 3.0, 2.1, and 4.7 percentage units compared to the controls for the 100R:0A, 67R:33A, 33R:67A, and 0R:100A treatments, respectively. However, site of infusion did not influence digestibility. In a study comparing ruminal vs. abomasal casein infusion on utilization of low-quality tall grass prairie forage, Bandyk et al. (2001) also reported increased OM digestion with casein infu-

sion but no difference between ruminal or abomasal infusion treatment. These data suggest that total-tract digestibility was not compromised as casein infusion was shifted from the rumen to the abomasum.

Implications

Protein supplementation decreased weight loss of lambs fed low-quality brome hay. Feeding protein supplements containing a portion (greater than 0%) of the crude protein as ruminally undegradable intake protein compared with 100% ruminally degradable intake protein to lambs consuming low-quality forage increases nitrogen retention and the efficiency of nitrogen utilization, without influencing total-tract nutrient digestion. These data suggest that because differences in nitrogen retention were minimal between lambs receiving from 33 to 100% of the supplemental protein abomasally,

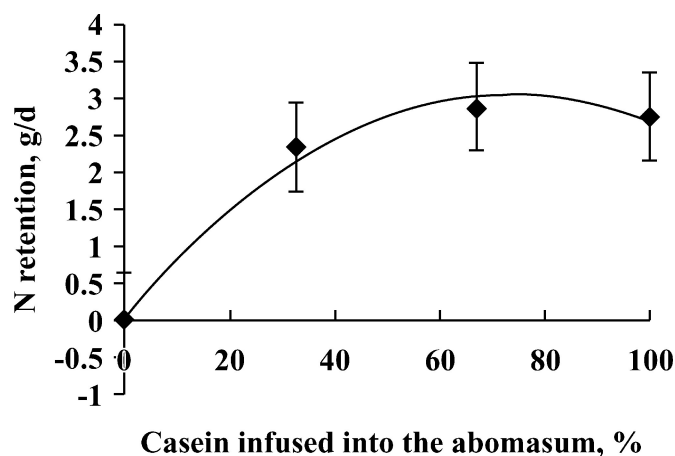


Figure 1. Influence of infusing different proportions of casein in the rumen and abomasum on N retention in lambs. Data are least square means and pooled SEM, $n = 4$. Quadratic regression curve was developed using all data points ($Y = -0.0006x^2 + 0.0816x + 0.1066$, $R^2 = 0.5221$).

differences in performance or nitrogen balance of ruminants fed low-quality forage supplemented with different protein sources may be the result of differences in protein quality rather than protein degradability.

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